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Attention Deficit/Hyperactivity Disorder and Childhood Autism in Association with Prenatal Exposure to Perfluoroalkyl Substances: A Nested Case–Control Study in the Danish National Birth Cohort

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Abstract

Background: Perfluoroalkyl substances (PFASs) are persistent pollutants found to be endocrine disruptive and neurotoxic in animals. Positive correlations between PFASs and neurobehavioral problems in children were reported in cross-sectional data, but findings from prospective studies are limited.

Objectives: We investigated whether prenatal exposure to PFASs is associated with attention deficit/hyperactivity disorder (ADHD) or childhood autism in children.

Methods: Among 83,389 mother-child pairs enrolled in the Danish National Birth Cohort during 1996–2002, we identified 890 ADHD cases and 301 childhood autism cases from the Danish National Hospital Registry and the Danish Psychiatric Central Registry. From this cohort, we randomly selected 220 cases of ADHD and autism each, and we also randomly selected 550 controls frequency matched by child's sex. Sixteen PFASs were measured in maternal plasma collected in early or mid-pregnancy. We calculated risk ratios (RR) using generalized linear models taking into account sampling weights.

Results: Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were detected in all samples; 4 other PFASs were quantified in ≥ 90% of the samples. We did not find consistent evidence of associations between mother's PFAS plasma levels and ADHD (per ln-ng/ml increase: PFOS RR = 0.87; 95%CI: 0.74, 1.02; PFOA RR = 0.98; 95%CI: 0.82, 1.16) or autism (per ln-ng/ml increase: PFOS RR = 0.92; 95%CI: 0.69, 1.22; PFOA RR = 0.98; 95%CI: 0.73, 1.31). We found positive as well as negative associations between higher PFAS quartiles and ADHD in models that simultaneously adjusted for all PFASs, but these estimates were imprecise.

Conclusions: In this study we found no evidence to suggest that prenatal PFAS exposure increases the risk of ADHD or childhood autism in children.

Introduction

Perfluoroalkyl substances (PFASs) are a group of man-made fluorine-containing compounds with unique properties making materials stain, oil, and water resistant (Buck et al. 2011). PFASs were broadly used in commercial products since the 1950s and they are persistent in the environment and in living organisms throughout the globe (Houde et al. 2006). Human exposure routes include contamination of food from packaging, bioaccumulation in the food chain, and household dust (D'eon and Mabury 2011). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two most frequently used PFASs with estimated biological half-lives in humans between 4 to 5 years (Olsen et al. 2007). PFOS and PFOA concentrations in humans were reported to be decreasing in some countries following a drop in production since 2000 (Kato et al. 2011), but exposure to other short-chain compounds such as perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) and long-chain perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) are reported to be increasing (Glynn et al. 2012).

PFASs can cross the placental barrier and expose the fetus during the most vulnerable period of development (Fei et al. 2007). Experimental data suggest that PFASs may be developmental neurotoxicants that can affect neuronal cell development (Slotkin et al. 2008), alter cognitive function, and reduce habituation and learning ability in mice (Johansson et al. 2008; Johansson et al. 2009; Viberg et al. 2013). PFASs also have endocrine disruptive properties (Kjeldsen and Bonefeld-Jorgensen 2013) and might interfere with thyroid hormone function (Lau et al. 2003; Lin et al. 2013; Long et al. 2013; Wang et al. 2014) which is essential in regulating fetal brain development (Porterfield 2000).

Attention deficit/hyperactivity disorder (ADHD) is considered one of the most common neurobehavioral disorders worldwide characterized by inattention, hyperactivity, increased impulsivity, and motivational/emotional dysregulation (Polanczyk et al. 2007). Autism is a neurodevelopmental disorder characterized by impairments in communication and reciprocal social interaction, coupled with repetitive behavior (Pickett and London 2005). The incidence of ADHD and autism has increased over the past decades, and it has been suggested that the rise is not solely attributable to changes in diagnostic practices or parental awareness (Faraone et al. 2003; Hertz-Picciotto and Delwiche 2009; Moller et al. 2007). The etiologies are not well understood but both environmental and genetic factors are thought to contribute to ADHD and autism (Millichap 2008; Lyall et al. 2014). ADHD and autism disproportionately affect boys (Arnold 1996), and studies suggest that prenatal exposure to endocrine disrupting chemicals may be associated with the occurrence of both diseases (de Cock et al. 2012).

A limited number of epidemiologic studies have evaluated the potential neurobehavioral or neurocognitive impact of PFASs and findings were inconclusive. Several cross-sectional studies have reported positive associations between serum levels of some PFASs with impulsivity (Gump et al. 2011) and ADHD in children (Hoffman et al. 2010; Stein and Savitz 2011). Reverse causality, however, is a concern for studies that measure PFAS levels in children already diagnosed with ADHD at time of blood draw. Little evidence of associations were found for prenatal exposures to PFOS or PFOA and behavioral problems in 7-year-old children assessed with the Strengths and Difficulties Questionnaires in the prospective Danish birth cohort (Fei and Olsen 2011). A study conducted in a community with high long-term exposure to PFOA in contaminated drinking water, reported that *in utero* PFOA levels were associated with higher Full Scale Intelligence Quotient (IQ) and decreased ADHD characteristics among children aged

6-12 years (Stein et al. 2013). However, prenatal PFOA exposures were estimated based on exposure modeling. A recent study examined the associations between several endocrine-disrupting chemicals, including PFASs, and autistic behaviors in children but no conclusive evidence was found, perhaps due to small sample size (175 mothers and children) and low statistical power (Braun et al. 2014).

We conducted a case-cohort study within the framework of the Danish National Birth Cohort (DNBC) to examine whether prenatal exposure to PFASs is associated with ADHD or autism in children.

Methods

The DNBC is a nationwide cohort study of pregnancies and health related outcomes in the children (details have been described elsewhere (Olsen et al. 2001)). Briefly, pregnant women were recruited through their general practitioners during early gestation (weeks 6 to 12) from 1996 to 2002. About 50% of all general practitioners in Denmark participated in the study, and 60% of the women invited agreed to participate. Women were ineligible if they did not speak sufficient Danish for interviews or intended not to carry their pregnancy to term. Information was collected in four computer-assisted telephone interviews (twice during pregnancy and twice postpartum). Two prenatal maternal blood samples were collected and stored, one each in the first and second trimester. English versions of questionnaires are available online (Access to DNBC data 2013).

Written informed consent was obtained from all participants at recruitment. Study procedures have been approved by the Danish Data Protection Agency, and the Institutional Review Board at University of California, Los Angeles.

Source population

The source population for this study consisted of live born singletons, and mothers who participated in the first telephone interview conducted approximately during the 12th gestational week and had provided a blood sample at least once either during the first or second pregnancy trimesters. This resulted in 83,389 mother-child pairs with 42,737 boys and 40,652 girls; we excluded from the original DNBC those with an unsuccessful pregnancy (n=6,207), non-singleton births (n=2,080), births with unknown birth outcomes (n=25) or missing dates of birth (n=99), mothers who emigrated (n=51) or died (n=3), and women who did not participate in the first telephone interview (n=4,578) or did not provide a prenatal blood sample (n=4,609).

Selection of cases and controls

We identified children who were diagnosed with ADHD and autism, respectively, by linking DNBC records to the Danish National Hospital Registry (Andersen et al. 1999) that contains the nationwide data for all admissions for somatic illnesses, and also to the Danish Psychiatric Central Registry (Munkjorgensen et al. 1993) which covers admissions to all psychiatric hospitals in Denmark. The record linkage relied on the unique civil registration numbers given to all Danish citizens at birth. All diagnoses are based on the International Classification of Diseases, 10th version (ICD10 F90.0 for ADHD; F84.0 for childhood autism) and included inpatients and outpatients records. A total of 890 ADHD cases and 301 autism cases were identified in the cohort during an average of 10.7 years of follow-up (record linkage was conducted on August 1st, 2011). Due to the high costs of measuring PFASs we randomly selected 220 cases of ADHD and autism each for inclusion in this study.

We randomly selected 550 children (440 males and 110 females) as controls from the source population, frequency matched to cases by sex. The flowchart of subject selection and sampling fractions of cases and controls are shown in Figure 1.

PFAS measurements

Details about analytic methods for PFASs have been described previously (Liew et al. 2014). Briefly, the collected maternal blood samples were sent by mail to Statens Serum Institut in Copenhagen, and separated and stored in freezers at -20°C, -80°C or in liquid nitrogen. We used 0.1 ml stored maternal plasma and the samples were analyzed at the Department of Environmental Science at Aarhus University. The majority of samples (87%) for both cases and controls were collected during the first trimester; if the first sample was not available we used the second sample collected in the second trimester instead. Solid Phase Extraction (SPE) technique was used for extraction and purification, and PFAS concentrations were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Measurements were performed in a random sequence for cases and controls by laboratory personnel blinded to diagnoses and any other participant information. Seventeen maternal samples (5 ADHD, 7 autism, 5 controls) were either not available from the biobank or failed the PFAS extraction and purification process hence were excluded. For 21 samples included in our current study PFOA and PFOS values previously had been analyzed at the 3M Toxicology Laboratory for an earlier study in the DNBC (Fei et al. 2007). For quality control we compared the PFOA and PFOS value measured from the two labs in these samples and found high correlations (Pearson correlation r=0.94 for PFOS and r=0.95 for PFOA).

Of the 16 different PFASs detected in maternal plasma, we focus on the 6 PFASs for which at least 90% of all samples were above the lower limit of quantitation (LLOQ): PFOS 100%, PFOA

100%, PFHxS 98%, PFHpS (perfluoroheptane sulfonate) 96%, PFNA 92%, PFDA 90%. The LLOQ for the 6 PFASs are as following: PFOS 0.28 ng/ml, PFOA 0.20 ng/ml, PFHxS 0.08 ng/ml, PFHpS 0.11 ng/ml, PFNA 0.27 ng/ml, and PFDA 0.09 ng/ml. The full panel for the LLOQ and distribution of all PFASs was reported previously (Liew et al. 2014).

Statistical analysis

We used generalized linear models and accounted for the sampling fractions of cases and controls to estimate risk ratios (RR) and 95% confidence intervals (CI) for prenatal PFAS exposures and ADHD or autism, respectively. We also performed unconditional logistic regression to estimate odds ratios (OR) without applying the sampling weights. PFAS concentrations were first analyzed as continuous variables (per natural-log unit increase). The PFAS values were natural-log transformed in order to reduce the influence of outliers, to improve the model fit, and to make interpretation simpler and more consistent across different PFASs that vary in their ranges of concentration. We also categorized PFAS values into quartiles according to the distribution among controls using the lowest quartile as the reference group. Moreover, we fitted generalized additive models with a smoothing function of natural-log PFAS values to examine potential non-linear relations. Five knots were set as the upper limit of number of degrees of freedom, and we compared model fit and visually inspected plots of the smoothed data. We did not find evidence for non-linearity between natural-log PFAS values and ADHD or autism (data not shown).

Potential confounders were chosen *a priori* considering variables that may influence PFAS exposures and previously suggested risk factors of ADHD or autism. We include maternal age at delivery (≤24, 25-29, 30-34, ≥35 years), parity (1, >1), socio-economic status (low/medium or high), maternal smoking (never, ≤9 cigarette/day, >9 cigarettes/day) and alcohol drinking (yes,

no) during pregnancy, mother's self-reported psychiatric illnesses (yes, no), gestational week of blood draw (4-8, >8 week), child's birth year (1998-2000, 2001-2003), and the matching factor child's sex in the final model. Socio-economic status was created based on self-reported maternal and paternal education and occupation using three categories (high, medium and low): higher education (four years beyond high school) or work in management were classified as high, skilled workers and middle-range education as medium, unskilled workers and unemployed as low (Bech et al. 2005). To determine maternal psychiatric illnesses, women were asked to report if they had ever seen a doctor or psychologist because of depression, anxiety, childhood psychiatric disorder, family problems/life crisis, or other mental health problems. Additionally, other potential confounders such as father's age at child's birth, mother's pre-pregnancy body mass index, whether the pregnancy was planned, and season of conception were evaluated but not included in final models since they changed effect estimates of interest minimally (<1%).

To account for PFAS values below the LLOQ when PFASs were analyzed as continuous variables, we used multiple imputation (Lubin et al. 2004) with the procedure "PROC MI" in SAS with all six PFASs and all covariates included in the model. Ten simulated complete datasets were generated via imputation, and we employed standard analytical procedures to combine the results (Yuan 2001).

A Pearson correlation matrix for the considered PFASs is presented in Supplemental Material, Table S1. We constructed a "multiple PFAS" model where we simultaneously included all PFASs in one model to examine whether any single PFAS may be of particular importance. We also evaluated potential effect measure modification by child's sex; we compared the sex-stratified estimates and examined the p-value for the PFAS–sex interaction term. For ADHD we also conducted analyses in which we excluded children born after the year 2000 because the

duration of follow-up may not have been long enough to identify children with this diagnosis. For these stratified analyses we used logistic regression without applying sampling weights because the weighted analyses may under-estimate uncertainty in our data when the number of actual measured samples is small. Finally, in sensitivity analyses we excluded PFAS values that were greater than three times the 75th percentile (n=2 PFOA, n=7 PFHxS, n=1 PFNA, n=2 PFHpS, n=1 PFDA) to ensure that individuals with extreme exposure values did not disproportionately influence our results. Analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Table1 presents the demographic characteristics of cases and controls. Table 2 shows the median and inter-quartile range distribution of maternal PFAS concentrations during pregnancy in cases and controls.

We generally found no association between ADHD or autism in children and PFAS levels in maternal plasma (modeled as natural log units) (Table 3). We did not detect apparent effect modification by child's sex (all PFASs and sex interaction p-values $\geq$0.25), but since both diagnoses were more prevalent in boys, estimates for girls were less precise (Supplemental Material, Table S2).

When we categorized PFAS values, mothers in the highest quartile of PFOS, PFHxS, PFHpS and PFDA were less likely to have a child diagnosed with ADHD than mothers in the lowest quartile after adjustment for potential confounders (Table 4). When all PFASs were simultaneously entered into the model, PFOA and PFNA levels were positively associated with ADHD, while negative associations with the other compounds persisted, with most showing monotonic trends.

There was some evidence of a positive association between PFHxS and autism, though RRs for the highest quartile were closer to the null than RRs for the second and third quartiles. Similar patterns were found with lower precision of the estimates when we used logistic regression without applying sampling weights (Supplemental Material, Table S3).

Results were similar to those from the primary models when we performed additional sensitivity analyses restricting the analyses to children born prior to 2001 (Supplemental Material, Table S4), and excluding extreme PFAS values (results not shown).

Discussion

Overall, our results do not suggest that prenatal exposure to PFASs increases the risk of ADHD or childhood autism in children. We observed some inverse associations between several PFASs and ADHD after controlling for potential confounders. In the "multiple PFAS" model, we found some positive as well as negative associations between PFASs and ADHD but these might be subject to multi-collinearity or sparse data bias. Results were mostly close to null for autism in both single and multiple PFAS models.

Toxicology studies raised concerns that PFASs are neurotoxic, hormonal disruptive, and can impair fetal brain development (Johansson et al. 2008; Lau et al. 2003; Long et al. 2013). However, some neurotoxic effects in rats were observed at doses several orders of magnitude higher than the PFAS levels found in the U.S. and Danish general population (Butenhoff et al. 2009; Fei et al. 2007). Several epidemiologic studies have investigated associations between PFASs and hyperactivity or behavioral problems in children, but the findings have been inconclusive (Braun et al. 2014; Fei and Olsen 2011; Hoffman et al. 2010; Stein and Savitz 2011; Stein et al. 2013). A previous study based on a subsets of children from the Danish National

Birth Cohort found some inverse-associations between prenatal PFOA, but not PFOS, and behavioral problems in 7-year-old children measured by (parent reported) items in the Strength and Difficulty Questionnaire (Fei and Olsen 2011). Another study also suggested a lower prevalence of ADHD characteristic in children associated with higher estimated in-utero PFOA exposures based on the Clinical Confidence Index (Stein et al. 2013). There is however no biologic explanation for PFASs protecting the developing brain from ADHD and potential biases such as uncontrolled confounding or selection bias might have driven these unexpected findings. No apparent associations were found between PFASs and autism in current and a previous small study (Braun et al. 2014).

Since several PFASs are moderately to highly correlated, it is difficult to disentangle mixture effects from compound-specific effects. A recent *in-vitro* assay reported an additive or more than additive antagonistic effect for a mixture of compounds (PFHxS, PFOS, PFOA, PFNA, and PFDA) on androgen receptor function (Kjeldsen and Bonefeld-Jorgensen 2013). Unfortunately our sample is too small to allow for interaction analyses between different PFASs. Further experimental studies are needed to determine mechanisms of action for PFAS mixtures on biologic targets that could better inform our population-based studies in terms of the most biologically relevant exposure model to be employed.

It has previously been shown that prenatal exposure to PFASs can increase the incidence of fetal resorption and neonatal deaths in animal models (Abbott et al. 2007; Lau et al. 2007; Luebker et al. 2005). PFASs may interfere with sex and thyroid hormone homeostasis (Kjeldsen and Bonefeld-Jorgensen 2013; Lin et al. 2013; Wang et al. 2014), and it has been suggested that higher PFASs level are associated with reduced fecundity in women (Buck Louis et al. 2013; Fei et al. 2009) and with an increased risk for miscarriage (Darrow et al. 2014). It is therefore

possible that PFASs exposure at a level that reduces fetal or neonatal survival, especially in high risk fetuses and infants susceptible to neurological disorders such as ADHD and autism, could appear to have null or even protective effects on adverse neurobehavioral outcomes in children based on observational studies, since only live-born children can be followed-up and examined.

There are several strengths in our study. First, the PFASs measures were obtained from maternal plasma samples collected in pregnancy prior to the assessment of the outcomes in the children. Previous studies have shown that PFASs are stable in human serum and measurements obtained from serum or plasma samples gave comparable results (Ehresman et al. 2007). High correlations between maternal and cord blood PFASs measures were also reported and suggested that PFASs measured in maternal plasma can be used as a reasonable surrogate for fetal exposure levels throughout gestations (Fei et al. 2007). Furthermore, the maternal PFAS levels in our study are similar to those previously measured during the same time period in the U.S. general population (Calafat et al. 2007). Study participants were selected from a well-defined nationwide pregnancy cohort with an average of 10.7 years of follow-up, sufficiently long to assess the outcomes of interest. The outcome measures were clinical diagnoses using standardized diagnostic criteria from both the general and psychiatric hospital registries in Denmark, a country with high quality health care and universal coverage for its population. Diagnoses of childhood autism recorded in the psychiatric registry have previously been shown to have high validity: a study extracted and reviewed the medical records of 499 childhood autism cases from the registry and confirmed the diagnoses for 94% of the cases (Lauritsen et al. 2010). Follow-up was conducted through record linkage that did not require subjects' responses, thus minimizing chances for selection bias due to subject's non-response.

Our report also has some limitations. Both ADHD and autism are about 4 times more prevalent in boys, and due to cost limitations we were required to sample no more than 220 cases for each diagnostic group resulting in few female cases (n=41 with ADHD; n=33 with autism). Thus, our subgroup analyses by sex were relatively imprecise for girls resulting in effect estimates with wide confidence intervals. For autism, the cases were limited to children diagnosed with childhood autism. While this is the most severe disorder of the autism spectrum, it constitutes of only a part of autistic spectrum disorders, specifically children with Asperger's syndrome and other pervasive development disorders were not studied. Moreover, we have no data for other endocrine disrupting chemicals, preventing us from evaluating possible correlations or interactions of PFASs with other ubiquitous environmental chemicals with these properties such as polychlorinated biphenyls (PCBs), organophosphates, bisphenol A, and phthalates (de Cock et al. 2012; Polanska et al. 2012). Further, our blood samples had to be transported to the lab by ordinary mail before being processed which may have induced some random measurement errors.

In summary, we found no consistent evidence that prenatal PFAS exposures were associated with ADHD or childhood autism in children in the Danish National Birth Cohort. Both weak negative associations as well as some positive associations between PFASs and ADHD we observed in multiple PFAS models should be further explored. It is recommended that future studies analyze a larger sample, consider both prenatal exposure and exposure during first year of life, assess the potential mixture effects of exposures to different co-occurring endocrine disruptors, and examine more sensitive indicators such as neuropsychological functioning in children.

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Table 1. Characteristic of study participants.

Characteristic[a]	ADHD (N=220)		Childhood autism (N=220)		Controls (N=550)	
	n	%	n	%	n	%
Child's sex						
Male	179	81.4	187	85.0	440	80.0
Female	41	18.6	33	15.0	110	20.0
Mother age at delivery (years)						
≤ 24	37	16.8	28	12.7	42	7.6
25-29	83	37.7	81	36.8	235	42.7
30-34	72	32.7	75	34.1	201	36.5
≥ 35	28	12.7	36	16.4	72	13.1
Socio-economic status						
Low / Medium	112	50.9	74	33.6	209	38.0
High	106	48.2	144	65.5	339	61.6
Parity						
1	107	48.6	119	54.1	247	44.9
>1	100	45.5	96	43.6	288	52.4
Maternal drinking during pregnancy						
No	79	35.9	79	35.9	161	29.3
Yes	141	64.1	141	64.1	389	70.7
Maternal smoking during pregnancy						
Never	139	63.2	142	64.5	409	74.4
≤ 9 cigarettes/day	32	14.5	33	15.0	64	11.6
> 9 cigarettes/day	49	22.3	45	20.5	77	14.0
Mother's self-reported psychiatric illnesses						
No	167	75.9	173	78.6	469	85.3
Yes	53	24.1	47	21.4	81	14.7
Child's birth year						
1998-2000	133	60.5	114	51.8	322	58.5
2001-2003	87	39.5	106	48.2	228	41.5
Gestational weeks at blood draw						
4-8 weeks	87	39.5	88	40.0	216	39.3
>8 weeks	119	54.1	115	52.3	305	55.5

[a]The missing values for socio-economic status, parity, and gestational weeks at blood draw are a bout 1%,4%, 7% respectively.

Table 2. Distribution of maternal plasma PFAS concentrations in cases and controls.

Perfluoroalkyl substances	Abbreviation	Carbon chain length[a]	Percentage quantifiable in all samples	PFAS concentrations in ng/ml (median; 25th, 75th)[b]		
				ADHD (N=215)	Childhood autism (N=213)	Controls (N=545)
Perfluorooctane sulfonate	PFOS	8	100%	26.80 (19.20, 35.00)	25.40 (18.73, 32.40)	27.40 (20.40, 35.60)
Perfluorooctanoic acid	PFOA	8	100%	4.06 (3.08, 5.50)	3.88 (3.08, 5.28)	4.00 (3.01, 5.42)
Perfluorohexane sulfonate	PFHxS	6	98%	0.84 (0.61, 1.15)	0.92 (0.70, 1.17)	0.92 (0.68, 1.23)
Perfluoroheptane sulfonate	PFHpS	7	96%	0.30 (0.20, 0.40)	0.28 (0.19, 0.38)	0.30 (0.21, 0.41)
Perfluorononanoic acid	PFNA	9	92%	0.42 (0.34, 0.52)	0.41 (0.33, 0.51)	0.43 (0.35, 0.56)
Perfluorodecanoic acid	PFDA	10	90%	0.15 (0.11, 0.20)	0.15 (0.11, 0.20)	0.17 (0.12, 0.23)

[a]The number of carbons in the fully fluorinated alkyl chain. [b]Concentrations for 17 samples (5 ADHD, 7 autism and 5 controls) were missing because the samples were either not available from the biobank or failed the extraction process.

Table 3. Risks ratios[a] for ADHD and childhood autism in children according to maternal plasma concentrations of PFAS in pregnancy.

	ADHD[b]		Childhood autism[b]	
Prenatal exposure	**Adjusted RR[c] (95% CI)**	**Adjusted RR[d] (95% CI)**	**Adjusted RR[c] (95% CI)**	**Adjusted RR[d] (95% CI)**
PFOS	0.87 (0.74, 1.02)	1.04 (0.70, 1.56)	0.92 (0.69, 1.22)	1.21 (0.69, 2.13)
PFOA	0.98 (0.82, 1.16)	1.21 (0.84, 1.74)	0.98 (0.73, 1.31)	1.15 (0.68, 1.93)
PFHxS	0.97 (0.88, 1.08)	1.05 (0.91, 1.20)	1.10 (0.92, 1.33)	1.26 (1.00, 1.58)
PFNA	0.80 (0.62, 1.03)	0.99 (0.58, 1.70)	0.80 (0.58, 1.11)	0.84 (0.48, 1.49)
PFHpS	0.91 (0.79, 1.05)	0.93 (0.64, 1.36)	0.91 (0.74, 1.12)	0.82 (0.56, 1.22)
PFDA	0.76 (0.64, 0.91)	0.80 (0.58, 1.11)	0.79 (0.63, 1.01)	0.82 (0.53, 1.28)

[a]Inverse probability weights derived from sampling fractions of cases and controls were applied in analyses. [b]215 ADHD cases, 213 autism cases, and 545 controls were used in analyses. [c]Adjusted for maternal age at delivery, SES, parity, smoking and drinking during pregnancy, psychiatric illnesses, gestational week of blood drawn, child's sex and birth year. [d]Adjusted for all covariates in c) additionally including all PFASs in the model.

Table 4. Risks ratios[a] for ADHD and childhood autism in children according to maternal plasma concentrations of PFAS (in quartiles) in pregnancy.

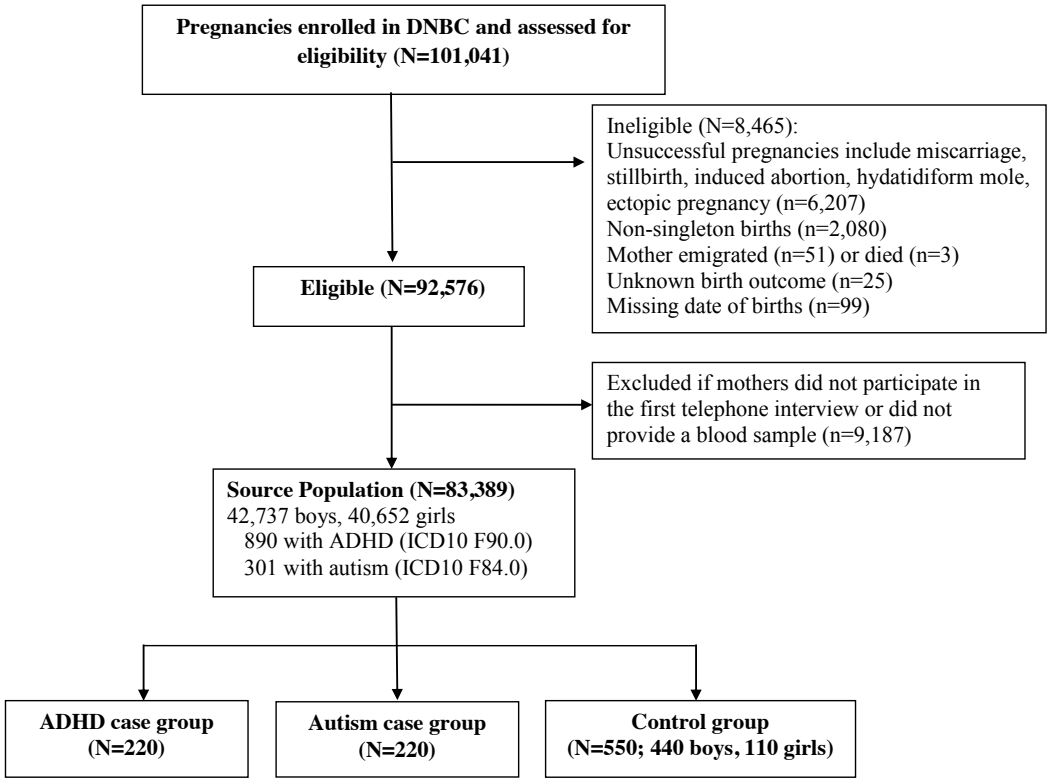
Prenatal exposure[b]	ADHD			Childhood autism		
	Crude RR	Adjusted RR[c] (95% CI)	Adjusted RR[d] (95% CI)	Crude RR	Adjusted RR[c] (95% CI)	Adjusted RR[d] (95% CI)
PFOS (ng/ml)						
3.85 - 20.40	1.00	1.00 (ref)	1.00 (ref)	1.00	1.00 (ref)	1.00 (ref)
20.41 - 27.40	0.83	0.95 (0.79, 1.15)	0.93 (0.75, 1.15)	0.72	0.91 (0.66, 1.25)	1.05 (0.73, 1.50)
27.41- 35.60	0.90	0.93 (0.76, 1.13)	0.86 (0.65, 1.12)	0.80	1.01 (0.73, 1.40)	1.20 (0.77, 1.89)
≥ 35.61	0.78	0.79 (0.64, 0.98)	0.65 (0.47, 0.91)	0.60	0.86 (0.59, 1.25)	1.16 (0.65, 2.09)
PFOA (ng/ml)						
0.57 - 3.01	1.00	1.00 (ref)	1.00 (ref)	1.00	1.00 (ref)	1.00 (ref)
3.02 - 4.00	1.00	1.02 (0.84, 1.23)	1.24 (0.99, 1.55)	1.05	1.13 (0.82, 1.56)	1.11 (0.76, 1.60)
4.01 - 5.42	1.13	1.09 (0.90, 1.33)	1.46 (1.14, 1.88)	1.03	1.05 (0.74, 1.47)	0.97 (0.63, 1.48)
≥ 5.43	1.07	1.14 (0.92, 1.40)	2.02 (1.49, 2.75)	0.78	0.95 (0.65, 1.38)	0.93 (0.54, 1.59)
PFHxS (ng/ml)						
<LLOQ - 0.68	1.00	1.00 (ref)	1.00 (ref)	1.00	1.00 (ref)	1.00 (ref)
0.69 - 0.92	0.97	1.05 (0.88, 1.26)	0.94 (0.76, 1.15)	1.26	1.33 (0.95, 1.87)	1.55 (1.06, 2.28)
0.93 - 1.23	0.90	0.94 (0.78, 1.14)	0.82 (0.65, 1.02)	1.38	1.50 (1.08, 2.10)	1.86 (1.25, 2.76)
≥ 1.24	0.64	0.67 (0.54, 0.83)	0.56 (0.43, 0.73)	0.94	1.07 (0.73, 1.56)	1.33 (0.84, 2.11)
PFNA (ng/ml)						
<LLOQ - 0.35	1.00	1.00 (ref)	1.00 (ref)	1.00	1.00 (ref)	1.00 (ref)
0.36 - 0.43	1.07	1.08 (0.90, 1.30)	1.29 (1.05, 1.59)	1.06	1.06 (0.78, 1.44)	0.94 (0.66, 1.34)
0.43 - 0.56	1.28	1.12 (0.93, 1.33)	1.48 (1.18, 1.86)	1.03	0.81 (0.59, 1.11)	0.73 (0.49, 1.08)
≥ 0.57	0.75	0.85 (0.69, 1.04)	1.58 (1.17, 2.13)	0.70	0.80 (0.56, 1.12)	0.98 (0.59, 1.63)
PFHpS (ng/ml)						
<LLOQ - 0.21	1.00	1.00 (ref)	1.00 (ref)	1.00	1.00 (ref)	1.00 (ref)
0.21 - 0.30	0.74	0.70 (0.58, 0.84)	0.67 (0.54, 0.83)	0.83	0.82 (0.60, 1.12)	0.70 (0.49, 1.01)
0.30 - 0.41	0.91	0.87 (0.72, 1.05)	0.86 (0.65, 1.13)	0.82	0.92 (0.66, 1.29)	0.83 (0.53, 1.31)
≥ 0.42	0.75	0.71 (0.58, 0.87)	0.81 (0.57, 1.15)	0.66	0.82 (0.57, 1.19)	0.80 (0.44, 1.48)
PFDA (ng/ml)						
<LLOQ - 0.12	1.00	1.00 (ref)	1.00 (ref)	1.00	1.00 (ref)	1.00 (ref)
0.13 - 0.17	0.91	0.82 (0.69, 0.97)	0.80 (0.66, 0.96)	1.04	0.93 (0.69, 1.25)	0.99 (0.72, 1.37)
0.18 - 0.23	0.83	0.87 (0.72, 1.05)	0.91 (0.73, 1.14)	0.98	1.07 (0.77, 1.47)	1.34 (0.92, 1.95)
≥ 0.24	0.51	0.53 (0.43, 0.66)	0.53 (0.40, 0.72)	0.50	0.52 (0.35, 0.77)	0.73 (0.43, 1.24)

[a]Inverse probability weights derived from sampling fractions of cases and controls were applied in analyses. [b]PFAS values below the lower limit of quantitation (LLOQ) were grouped in the lowest quartile. [c]Adjusted for maternal age at delivery, SES, parity, smoking and drinking during pregnancy, psychiatric illnesses, gestational week of blood drawn, child's sex and birth year. [d]Adjusted for all covariates in c) additionally including all PFASs in the model.

Figure Legend

Figure 1. Flow chart of study population selection in the Danish National Birth Cohort. Sampling fraction of ADHD cases is 0.2472. Sampling fraction of Autism cases is 0.7309. Sampling fractions of control group are 0.0103 for male and 0.0027 for female.

Figure 1.


Pregnancies enrolled in DNBC and assessed for eligibility (N=101,041)
Ineligible (N=8,465):
Unsuccessful pregnancies include miscarriage, stillbirth, induced abortion, hydatidiform mole, ectopic pregnancy (n=6,207)
Non-singleton births (n=2,080)
Mother emigrated (n=51) or died (n=3)
Unknown birth outcome (n=25)
Missing date of births (n=99)
Eligible (N=92,576)
Excluded if mothers did not participate in the first telephone interview or did not provide a blood sample (n=9,187)
Source Population (N=83,389)
42,737 boys, 40,652 girls
890 with ADHD (ICD10 F90.0)
301 with autism (ICD10 F84.0)
ADHD case group (N=220)
Autism case group (N=220)
Control group (N=550; 440 boys, 110 girls)